Exhibit 10

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Potential of p38 MAP kinase inhibitors in the treatment of cancer

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Summary

The involvement of chronic inflammation in tumor development and progression is reviewed. Based on the natural history of certain diseases and epidemiology studies, a strong association has been established between particular chronic inflammatory conditions and eventual tumor appearance. Solid tumors require a stroma for their growth and recruit macrophages to synthesize essential growth and angiogenic factors that they do not have the capacity to produce. The microenvironment of the local host tissue appears to be an active participant in exchanging cytokines and enzymes with tumor cells that modify the local extracellular matrix, stimulate migration, and promote tumor angiogenesis, proliferation and survival. The role of p38 MAP kinase as a therapeutic target for treating cancer is discussed.

Contents

Key words

Tumor therapy, p38 mitogen-activated protein (MAP) kinase, angiogenesis, chronic inflammation, tumor necrosis factor-α, macrophage, metastatic activity.

Glossary of abbreviations

MAP, mitogen-activated protein; TNF-α, tumor necrosis factor-α; MMP, matrix metalloproteinase; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; EGF, epidermal growth factor; TPA, 12-O-tetradecanoylphorbol-13-acetate; IL-1, interleukin-1; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor; ICAM-1 intercellular adhesion molecule-1; CHO, Chinese hamster ovary; NSAID, nonsteroidal anti-inflammatory drug and MK2 or MAPKAP kinase 2, MAP kinase-activated protein kinase 2.

> *"Inflammation in itself is not to be considered as a disease, but as a salutary operation, consequent, either to some violence or to some disease. But this same operation can and does go vary; it is often carried further even in sound parts...*
> *Where it can alter the diseased mode of action, it likewise leads to a cure; but where it cannot accomplish that salutary purpose, as in cancer, scrofula, venereal disease, etc. it does mischief."*
>
> John Hunter (1794)

1 Introduction

The links between chronic inflammation and cancer are starting to have implications for cancer prevention and treatment. The existence of chronic inflammatory conditions that do not have an established infective cause and are associated with the development of tumors strongly suggests that the inflammatory process itself provides the prerequisite environment for the development of malignancy. The capacity for tumors to attract macrophages is central to the contention that macrophages and other inflammatory cells promote tumor emergence and that some host inflammatory responses encourage tumor growth and angiogenesis. It is possible that these intratumoral inflammatory cells and the local production of cytokines in the tumor microenvironment are more likely to contribute to tumor growth, progression, metastatic spread, and immuno-suppression than they are to mount an effective response against the tumor. It has been suggested that: "If genetic damage is the match that lights the fire of cancer, some types of inflammation may provide the fuel that feeds the flames" [1]. This review attempts to review the literature for the involvement of inflammatory processes in promoting tumor development and growth and provides a rationale for the use of p38 MAP kinase inhibitors in the prevention and/or treatment of malignant diseases. Evidence is presented to support the important role of p38 MAP kinase in controlling tumor vascularization, invasion, and metastatic spread.

2 Association of inflammation with cancer

The association of chronic inflammation with certain malignancies has been recognized for many years. In 1863, Rudolf Virchow suggested that "lymphoreticular infiltrate" reflected the origin of cancer at sites of chronic inflammation [2]. Chronic irritation, previous injuries, and ulcers were suggested to predispose for tumor development. These early observations on the connection between chronic inflammation and subsequent malignancy have been confirmed in the current medical literature. Tumor development at chronic inflammatory sites has now been observed clinically for a multitude of tissues (Tab. 1). In certain situations, such as tobacco exposure in lung cancer or head and neck cancer, where there appears to be an environmental car-

Table 1.
The association between chronic inflammation and cancer.

Cancer	Inflammatory stimulus/condition
Colorectal	Ulcerative colitis, Crohn's disease [3–5]
Colon	*Schistosoma japonicum* [6]
Esophageal	Reflux esophagitis or Barrett's metaplasia [7, 8]
Pancreatic	Chronic pancreatitis [9]
Gall bladder	Chronic cholecystitis and cholelithiasis [10]
Gastric	*Helicobacter pylori*-induced [11] and atrophic gastritis [12]
Urinary bladder	*Schistosoma haematobium* [13] and long-term indwelling catheterization [14]
Mesothelioma	Asbestos [15]
Hepatocellular	Hepatitis virus (B and C) [16, 17]
Cholangiocarcinoma	*Opisthorchis viverrini* [18]
Biliary tract cancer	*Chlonorchis sinensis* [18]
Cervical	Papillomavirus [19, 20]
Ovarian	Pelvic inflammatory disease [21]
Hodgkin's lymphoma	Granulomatous inflammation [22]
Skin	Chronic skin ulcers [23, 24]
Karposi's sarcoma	Human herpesvirus type 8 [25]
Burkitt's lymphoma	Epstein-Barr virus [26]
Naso-pharyngeal	Epstein-Barr virus [27]
Lung	Bronchitis from smoking [28]

cinogen, there are also likely to be chronic inflammatory components that contribute to cancer development. Chronic bronchitis and emphysema due to cigarette smoking are recognized risk factors for the development of lung cancer [28]. In addition, surgical wounds and associated inflammation may provide a favorable condition for tumor recurrence. This may lead to early tumor recurrence following primary resection of the cancer or tumor growth after invasive diagnostic procedures in cancer patients [29].

In addition to these clinical observations, several animal studies have demonstrated the contribution of inflammation to tumor development (reviewed in [29, 30]). For example, tumors in chickens induced by Rous sarcoma virus (RSV) preferentially develop at sites of injury and inflammation, despite systemic viral infection [31–33]. Granuloma formation by local injection of *bacillus Calmette-Guerin* into chick wing 7 days prior to RSV inoculation stimulated formation of RSV-induced tumors [34]. Similarly, tumors induced in rodents by systemically applied carcinogens appear preferentially at sites of wounding and inflammation [35, 36]. In addition, inflammation augments tumor formation in the mammalian skin, colon, and urinary blad-

der [37–43]. Transgenic mice carrying the *v-jun* oncogene developed dermal fibrosarcomas after full thickness wounding, whereas identical wounds in non-transgenic mice healed normally without tumor formation [44]. Transgenic mice carrying the *tat* gene from human T-cell lymphotrophic retrovirus developed mesenchymal tumors in areas prone to scratching [45]. Collectively, these studies demonstate the significant role of the microenvironment on tumor development.

3 Role of inflammation in multi-stage carcinogenesis

Cancer arises as a result of a multi-step process beginning from the initial benign transformation of cells and progressing to overt invasive, metastatic disease [46–48]. This process takes many years to progress and the length of time required strongly suggests that it takes place against a background of rigorous controls aimed at preventing anarchic cell behavior that threatens the life of the individual organism. The major stages of carcinogenesis have been termed initiation, promotion, and progression [49]. Tumor initiation begins when DNA in a cell is damaged by exposure to exogenous or endogenous carcinogens. If this damage is not repaired, it can lead to genetic mutations. The responsiveness of the mutated cells to their microenvironment can be altered and may give them a growth advantage relative to normal cells.

In the mouse two-stage skin carcinogenesis model, tumor promotion is a distinct, rate-limiting step that determines the formation of premalignant tumors. The two-stage model of experimental carcinogenesis involves sequential application of, first, a transforming agent and later, a tumor-promoting agent such as 12-O-tetradecanoylphorbol-13-acetate (TPA), the active principle of croton oil. The promoter itself is not carcinogenic, but induces intense infiltration with inflammatory cells and epithelial proliferation. However, epithelial proliferation in the absence of inflammation does not promote carcinogenesis [50, 51]. Moreover, both steroidal and nonsteroidal anti-inflammatory agents suppress tumor promotion by TPA in the mouse skin [52]. Schultz and coworkers presented evidence that macrophage secretions (e.g., angiogenesis-stimulating factors, prostaglandins, and clonal proliferation factors for carcinogen-triggered cells) may be involved in the tumor promotion process [53]. Tumor promoters generally increase the production of a number of growth factors and proinflammatory cytokines (such as tumor

necrosis factor-α (TNF-α), GM-CSF, IL-1, IL-6 and IL-8) and nonprotein factors (such as nitric oxide and other oxygen radicals) involved in inflammation and carcinogenesis [54, 55]. Studies of rodent tumor models of liver, bladder, colon and breast cancers – and the association of human cancer with inflammatory conditions (Tab. 1) – suggest that processes analogous to tumor promotion in mouse skin are a common feature of carcinogenesis (reviewed in [56]). Indeed, chronic inflammation, resulting from infective and/or non-infective agents, may provide the ideal environment for the development of the cell changes that lead to cancer [57, 58].

The study of tumor promotion in rodent carcinogenesis using chemical tumor promoters has revealed various tumor promotion pathways, such as the TPA pathway mediated through activation of protein kinase C, and the okadaic acid pathway mediated through inhibition of protein phosphatases 1 and 2A. Both TPA and okadaic acid induce TNF-α gene expression in mouse skin [59]. Moreover, Komori and associates demonstrated that TNF-α stimulated transformation of BALB/3T3 cells initiated with 3-methylcholanthrene 1,000-times stronger than did TPA [60], suggesting that TNF-α acts as an endogenous tumor promoter and plays a role in tumor promotion and progression in humans. Fujiki and Suganuma proposed a practical method of screening for chemical tumor promoters based on their induction of TNF-α release from HL-60 cells [59]. Mice deficient in TNF-α are resistant to skin carcinogenesis [61]. TNF-$\alpha^{-/-}$ mice were resistant to development of benign and malignant skin tumors, whether induced by initiation with DMBA and promotion with TPA or by repeated dosing with DMBA. TNF-$\alpha^{-/-}$ mice developed 5–10% of the number of tumors developed by wild-type mice during initiation/promotion and 25% of those in wild-type mice after repeated carcinogen treatment. The early stages of TPA promotion are characterized by keratinocyte hyperproliferation and inflammation. These were both diminished in TNF-$\alpha^{-/-}$ mice. The authors noted that strategies that neutralize TNF-α production may be useful in cancer treatment and prevention.

Aside from producing various growth and angiogenic factors for tumor development, inflammatory cells can increase genotoxic damage in tissue by at least three mechanisms. The cells release reactive oxygen species such as superoxide anion and hydrogen peroxide, as well as lipid oxidation products [62]. Inflammatory cells have also been shown to participate in the metabolic activation of procarcinogens to DNA-damaging species [63, 64]. For example, neutrophils activate aromatic amines, aflatoxins, estrogens, phenols, and

polycyclic aromatic hydrocarbons by oxidant-dependent mechanisms [63]. Finally, studies implicate inflammatory cells in the formation of carcinogenic nitrosamines [65].

4 Tumors as wounds that do not heal

Solid tumors consist of two discrete but interdependent compartments: the malignant cells themselves and the stroma that they induce and in which they are dispersed [29, 66, 67]. All solid tumors, regardless of their site of origin, require stroma if they are to grow beyond a minimal size of 1 to 2 mm [68]. Seljelid and associates suggested that the stromal cells, such as macrophages, may be considered "slaves", kept to carry out certain functions, synthesize essential substances (e.g., growth and angiogenic factors) that the tumor cells do not have the capacity or the degree of finely tuned machinery to produce [67]. Tumor stroma consist of new blood vessels, inflammatory cells (primarily macrophages and lymphocytes), connective tissue, consisting of matrix components, such as fibronectin, interstitial collagens, elastin and glycosaminoglycans, and fibrin-gel matrix. Dvorak suggested that tumor stroma generation is wound healing gone awry [69]. He argued that successful tumors – that is, tumors that grow progressively in the host – are obligate parasites. They subvert the wound-healing response as a means to acquire the stroma that they need to grow and expand. Tumors mimic wounds by depositing an extravascular fibrin-fibronectin gel, which signals the host to marshal the wound-healing response. Tumors constitutively secrete a vascular permeability factor that renders local blood vessels permeable to plasma proteins for protracted periods. As a result, persistent extravasation of fibrinogen and fibronectin around tumors and the continuous generation of new provisional matrix appear as an unending series of wounds that continually initiate healing but never heal completely.

There are numerous similarities between wound and tumor stroma [29, 69]. Plasma fibrinogen is rapidly transglutaminated to cross-linked fibrin in tumors, which has a striking parallel with healing wounds in which deposition and cross-linking of fibrin is an early event. As is typical for wounds, tumor growth causes the disorganization of fibroblasts and loss of contact inhibition, leading to proliferation, release of a variety of growth factors, which may include fibroblast growth factor (FGF), epidermal growth factor

(EGF) and transforming growth factor-β (TGF-β) and augmented secretion of extracellular matrix components. Other characteristics common to the environments of wounds and tumors include the presence of angiogenic vasculature and infiltration by inflammatory cells with the concomitant secretion of inflammatory mediators [29].

Additional studies provide evidence that wounds create an environment that is beneficial to tumor growth and possibly increases the metastatic potential of local tumor cells. For example, wounding of Rous sarcoma virus-infected chickens led to tumor formation with 100% frequency at the injured site, which would otherwise remain tumor-free [33]. A surgical wound also provides a favorable condition for tumor recurrence. The site of anastomosis after partial gastric resection or low anterior resection provided a preferential site for tumor recurrence [70–72].

5 Involvement of inflammation in tumor angiogenesis

Cancer growth and spread depends on the establishment of an adequate blood supply. Angiogenesis, the formation of new blood vessels, is an important event in a variety of physiological settings, such as embryonic development, chronic inflammation, and wound repair. Folkman hypothesized that tumors are angiogenesis-dependent [73]. This idea can be simply stated: Once a tumor is established, every increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor. The crucial significance of tumor angiogenesis as a target for anticancer therapy is underscored by the fact that most tumors would not develop beyond 2–3 mm^3 and could not metastasize without vascularization [74, 75].

Jackson and coworkers reviewed the literature that angiogenesis and chronic inflammation are codependent [76] and suggested the potential value of targeting chronic inflammation to affect angiogenesis. The link between angiogenesis and chronic inflammation involves both augmentation of cellular infiltration and proliferation and overlapping roles of regulatory growth factors and cytokines. The signals controlling angiogenesis, while directed at endothelial cells, come from cells in the nearby tissues, including tumor cells, keratinocytes, fibroblasts, monocytes, and macrophages [74, 76]. Of these cells, the inflammatory monocyte/macrophage cell type appears to play a key role in inflammatory and tumor angiogenesis. Acti-

vated monocytes and/or macrophages alone are sufficient to induce angiogenesis in the avascular cornea [77]. Moreover, almost every growth factor and cytokine known to regulate angiogenesis can be produced by macrophages [78]. *In vitro* studies have shown that macrophages produce in excess of 20 molecules that reportedly influence endothelial cell proliferation, migration, and differentiation *in vitro*, including VEGF, bFGF, TGF-α, TNF-α, IGF-1, EGF, platelet-derived endothelial growth factor/thymidine phosphorylase, IL-8, and IL-10. Moreover, macrophages are able to modulate events in the extracellular matrix either via the direct secretion of degradative enzymes or via extracellular matrix-modulating cytokines, such as collagenase, TGF-β, tissue-type plasminogen activator, plasminogen activator inhibitor-1, platelet-derived growth factor, angiotropin, and IL-6. By release of these factors, activated macrophages have the capability to influence each phase of the angiogenic process, such as alterations of the local extracellular matrix, induction of endothelial cells to migrate and proliferate, and inhibition of vascular growth with formation of differentiated capillaries.

Indeed, tumor-associated macrophages have been shown to induce neovascularization in the rat cornea *in vivo* and endothelial proliferation *in vitro* [79]. Mice depleted of macrophages by whole body x-irradiation or azathioprine administration before or after implantation of a syngeneic fibrosarcoma demonstrated a delay in the appearance of tumors, a suppression in the growth of established tumors, and a marked reduction in tumor vascularization [80, 81]. In addition, macrophages may promote tumor growth when admixed with tumor inocula [82, 83]. Several investigators noted that vascularization of human tumor cell lines grown on the chorioallantoic membrane of the chick embryo or subcutaneously in nude mice occurred coincidentally with macrophage infiltration at the tumor site [84, 85]. These authors speculated that tumor growth might be partially dependent upon the angiogenic activity of infiltrating macrophages. Richter and colleagues noted that IL-10 suppresses tumor growth of certain tumors by inhibiting infiltration of macrophages which may provide tumor growth-promoting activity [86]. In addition, macrophages cultured in a low-oxygen environment have been shown to exhibit enhanced angiogenic activity *in vivo* [87]. The low-oxygen environment of tumors might serve to enhance macrophage-mediated angiogenic activity. Tumor growth appears to be dependent in part on the relative concentrations of pro-angiogenic and angiostatic mediators produced by tumor cells and tumor-associated host cells, including macrophages

[88]. Macrophages can produce substances that suppress angiogenesis, such as interferon-β [89] and thrombospondin-1 [88]. It appears that macrophages that fail to switch from a pro-angiogenic to an angio-inhibitory phenotype contribute to pathological angiogenesis.

Increased numbers of macrophages within tumors are closely related to tumor angiogenesis and poor prognosis in a wide variety of human cancers including breast, colorectal, and lung carcinoma and melanoma [90–95]. The tumors produce monocyte chemoattractant protein-1 (MCP-1), which recruits macrophages to the tumor site [96]. MCP-1 has been demonstrated to directly induce inflammatory angiogenesis in the rabbit cornea assay [97]. Studies aimed at inhibiting macrophage recruitment and deposit in tumors may be a useful strategy to limit tumor angiogenesis. For example, linomide treatment inhibited tumor-associated macrophage numbers and selectively inhibited their ability to secrete TNF-α, but not GM-CSF [98]. In addition, this treatment reduced blood vessel density in Dunning R-3327 MAT-Lu rat prostate tumors by 44%, and inhibited tumor growth by 69%.

Macrophage-induced angiogenesis is primarily mediated by TNF-α [99]. Administration of anti-IL-8 or anti-vascular endothelial growth factor (VEGF) antibody blocked TNF-α-induced neovascularization in the rabbit cornea *in vivo* [100], demonstrating the cascade of angiogenic factors initiated by TNF-α treatment. TNF-α induces a marked increase in MCP-1 production by tumor cells [101], which can further recruit macrophages into the tumor site. In addition, TNF-α up-regulates expression of the potent angiogenic factors VEGF, IL-8, and basic fibroblast growth factor (b-FGF) through activation of transcription factors in vascular endothelial cells and other cell types including tumor cells [102]. Not only the production of TNF-α [103], but many of the proangiogenic effects of TNF-α are controlled by p38 MAP kinase. For example, TNF-α-induced MCP-1 production is regulated by p38 MAP kinase [104]. Similarly, p38 MAP kinase activation by VEGF mediates actin reorganization and cell migration in human endothelial cells [105]. Besides TNF-α, the production of IL-1, IL-6, IL-8 and GM-CSF in macrophage is also regulated by p38 MAP kinase [106]. P38 MAP kinase also regulates monocyte differentiation and chemotaxis [107]. Both ERK 1/2 and p38 MAP kinase are requisite for the signal transduction of bFGF in endothelial cells [108]. SB220025, a selective inhibitor of p38 MAP kinase, has been demonstrated to prevent granuloma development in a murine air pouch angiogenesis model [109]. Thus, p38 MAP kinase is an important target in tumor angiogenesis by pre-

venting TNF-α production and the subsequent cascade of angiogenic factors and effects initiated by the tumor-associated macrophage. P38 MAP kinase has also been demonstrated to play an essential role in placental angiogenesis and a more general role in embryonic angiogenesis [110].

6 Effect of proinflammatory cytokines in metastasis

Several studies suggest that proinflammatory cytokines, including TNF-α and IL-1β, have activities that contribute to metastatic tumor spread. Orosz and coworkers showed that administration of recombinant mouse TNF-α 5 h before or 1 h after, but not 24 h after intravenous inoculation of fibrosarcoma cells caused an increase in tumor cell colony formation on the lung surface [111]. Moreover, they showed that antibody neutralization of endogenous tumor-induced TNF-α led to a significant decrease in the number of pulmonary metastases. In a subsequent study, they observed that administration of recombinant murine TNF-α in mice promoted liver metastasis formation in mice injected i.v. or s.c. with a highly metastatic subline of methylcholanthrene-induced lymphoma (ESb) [112]. Consequently, TNF-α-treated animals revealed a higher mortality. Several studies indicate that transfection of TNF-α in tumor cells confers a metastatic phenotype [113–115]. Implantation of TNF-α gene-transfected ESb tumors in syngeneic mice produce reduced survival rates that correlate with enhanced metastasis [113, 114]. Malik and coworkers showed that Chinese hamster ovary cells transfected with the gene for human TNF-α caused a greatly enhanced ability to invade peritoneal surfaces and metastasize in nude mice compared with cells transfected by the vector alone [115]. Wu and colleagues presented data suggesting that isolation stress-augmented TNF-α was involved in the enhancement of tumor invasion and metastasis of colon 26-L5 carcinoma cells [116]. IL-1 has similarly been shown to enhance metastasis formation [117, 118], and IL-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases [119]. Dong and associates presented evidence that the host environment promotes the constitutive activation of nuclear factor-κB and proinflammatory cytokine expression during metastatic tumor progression of murine squamous cell carcinoma [120]. Human clinical studies have similarly associated increased levels of TNF-α with metastatic disease [121–123].

6.1 Mechanisms for prometastatic effect

The pathogenesis of cancer metastasis consists of a series of sequential, interrelated steps that include dissociation of single tumor cells from the primary tumor, invasion of the surrounding extracellular matrix including basement membranes, penetration of vascular and lymphatic spaces, escape from immunological control, arrest in distant organs, extravasation from the vascular or lymphatic circulation, and finally propagation into the target tissue and proliferation as a secondary colony [124]. Each step in the pathogenesis of metastasis is rate-limiting, and failure to complete any one prevents the malignant cells from producing a metastasis [125]. Since failure may occur at any step, only a very small percentage of tumor cells will finally survive and form metastases. Another crucial step in the progressive growth of primary neoplasms and metastasis is vascularization of the tumor and its surroundings [126].

There are several actions of TNF-α and IL-1 that could theoretically contribute to promotion of tumor spread. These include the induction of proteolytic enzymes that degrade extracellular matrix ([116, 127–129]; also Section 6.2), promotion of adhesion of tumor cells to endothelial cells [112, 130–135], stimulation of angiogenesis ([136]; also Section 5), stimulation of tumor cell motility [137], impairment of natural killer activity [138], stimulation of bone resorption [139], and induction of stromal proliferation [140].

The interaction of neoplastic cells with extracellular matrix protein by specific cell surface receptors is considered an important step in the invasion process, a critical phase in the complex chain of events leading to metastasis [141]. Experimental evidence from several different models suggests that enhanced adhesion of tumor cells to endothelial cells by TNF-α or IL-1 is an important underlying mechanism for enhancement of tumor metastases. The endothelial adhesive molecules of the ICAM and ELAM type are induced by cytokines, mainly TNF-α and IL-1. Blocking of integrin-matrix interactions with monoclonal antibodies or competing peptides inhibited tumor cell adhesion to endothelioma cells *in vitro* and lung colony formation of tumor cells *in vivo* [130, 131]. Miele and coworkers showed that the enhanced metastatic ability of TNF-α-treated malignant melanoma cells is reduced by intercellular adhesion molecule-1 (ICAM-1) antisense oligonucleotides [132]. Miyata and colleagues demonstrated that the metastasis-enhancing potential of human TNF-α could be overcome by introducing the cell-adhesive Arg-

Gly-Asp sequence [134]. It is interesting to note that SB203580, a selective p38 MAP kinase inhibitor, suppresses TNF-α-induced surface expression of the endothelial adhesion molecule VCAM-1 [142]. VCAM-1 mRNA accumulation induced by TNF-α was not affected by SB203580, suggesting that the p38 MAP kinase signaling cascade regulated the endothelial expression of VCAM-1 at the post-transcriptional level. Arachidonic acid has been demonstrated to activate MAPKAP K2 and mediate adhesion of human breast carcinoma cells to collagen type IV through a p38 MAP kinase-dependent pathway [143]. Hayes and Bergan showed that p38 MAP kinase is required for TGF-β-mediated cell adhesion in metastatic prostate cancer cells [144]. Laferriere and colleagues noted that transendothelial migration of colon carcinoma cells requires expression of E-selectin by endothelial cells and activation of p38 MAP kinase [145]. The ability of p38 MAP kinase inhibitors to regulate the expression of adhesion molecules on tumor and endothelial cells may be an important target for preventing metastasis formation.

6.2 p38 and the "invasive" tumor phenotype

Tumor invasion is an important step in the sequential process of metastasis. It basically consists of three steps: the attachment of invading tumor cells, proteolytic degradation of extracellular matrix, and active movement of tumor cells into the area of matrix lysis. The degradation of extracellular matrix by proteinases, such as urokinase-type plasminogen activator and matrix metalloproteinase (MMP), secreted by different cell types participating in tumor invasion such as macrophages and tumor cells, is considered to be a crucial event for tumor invasion and subsequent metastasis [124, 141, 146-148]. The expression of MMPs and urokinase-type plasminogen activator is transcriptionally regulated by a variety of factors including cytokines and growth factors, which are derived from macrophage and other tumor-infiltrating inflammatory cells, stromal cells, and tumor cells [147, 149].

Tumor-derived serine proteinases and MMPs have been associated with invasion and metastasis of cancer cells. Several investigators have recently noted the link between p38 MAPK and the invasive phenotype of cancer. Chen and colleagues noted that α_v integrin, p38 MAPK, and urokinase-type plasminogen activator are functionally linked in invasive breast cancer [150]. They noted that blocking α_v integrin functionality with a function-blocking

monoclonal antibody or down-regulating α_v integrin expression with α_v-specific antisense oligonucleotides significantly decreased the levels of active p38 MAPK and inhibited cell-associated urokinase-type plasminogen activator expression in invasive breast cancer MDA-MB-231 cells. They also found that vitronectin/α_v integrin ligation specifically induced p38 MAPK activation and urokinase-type plasminogen activator up-regulation in invasive MDA-MB-231 cells but not in non-invasive MCF7 cells. Higher p38 MAPK activity may be important for breast cancer invasiveness by stabilizing urokinase-type plasminogen activator mRNA [151]. In MDA-MB-231 cells, TNF-α up-regulates IL-6, IL-8 and MMP 9, 1 and 13 are important in extracellular matrix degradation [129]. A novel vaccine therapy (TNF Autovac) was developed to block TNF by inserting foreign antigen epitopes within the TNF peptide and so stimulate a T helper and hence B cell response not only to the foreign epitope but also to the self protein, TNF. They vaccinated C57Bl/6 mice with TNF Autovac and produced 100-fold antibody response to TNF. The TNF Autovac reduced the number and size of metastases in the B16F10 murine metastases model. Reunanen and colleagues demonstrated that activation of p38α MAPK by TNF-α induces collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) expression in fibroblasts via mRNA stabilization [152]. Kawashima et al. noted that up-regulation of MMP-9 (type IV collagenase) together with enhanced motility and endothelial adhesion contribute to the increased metastatic ability of human osteosarcoma cells induced by TNF-α treatment [128]. Simon and associates demonstrated that the p38 MAPK pathway regulates the expression of the MMP-9 collagenase via AP-1-dependent promoter activation [153]. They showed that the selective p38 MAPK inhibitor, SB203580, reduced MMP-9 expression/secretion and *in vitro* invasion of cancer cells. SB203580 also reduced matrigel invasion and MMP-2 expression of malignant melanoma cells [154]. Jung and colleagues demonstrated that the p38 MAPK pathway is involved in urokinase plasminogen activator expression and matrix invasiveness in gastric cancer cells [155]. Collectively, these reports suggest that inhibitors of p38 MAPK may be promising substances to interfere with a signaling cascade associated with tumor cell invasion.

Huang and coworkers presented evidence that MMP-9 production by host macrophages promotes growth and invasion of xenografted ovarian cancer cells in nude mice [156]. They implanted MMP-9-expressing human ovarian tumor cells into both wild-type and MMP-9-null mice and showed that mice lacking the gene for MMP-9 produced far fewer and smaller tumors, as well

as less ascites fluid. However, if MMP-9-null mice were reconstituted with spleen cells – a rich source of macrophages – from wild-type mice, the growth of peritoneal tumors and the formation of ascites were greatly enhanced, and the microvessel density in these tumors was significantly higher. These studies further demonstrate the significant role of the local microenvironment in regulating the invasive and malignant behaviors of cancer cells. The authors suggested that MMP-9 and its source, the peritoneal macrophage, are potential selective targets for therapeutics in ovarian cancer.

7 Inflammatory cytokines and growth promotion

Inflammatory cytokines, such as TNF-α, IL-1, and IL-6, can directly enhance neoplastic development acting as autocrine or paracrine growth factors [157]. The role of IL-6 in myeloma growth is one of the most quoted examples of a cytokine-mediated autocrine stimulation of cancer. IL-6 is important for the growth of murine plasmacytomas and human myelomas (reviewed in [158]). Pristane-induced granulomas, which produce high concentrations of IL-6, are critical not only for induction but also for early transplantation of mouse plasmacytomas. Kawano presented evidence that IL-6 is a possible autocrine growth factor for human myeloma cells including a human myeloma cell line U266 [159]. These myeloma cells express the IL-6 receptor, produce IL-6, and their in vitro growth is inhibited by antibodies to IL-6. In a similar study, Klein and coworkers were unable to confirm the autocrine hypothesis in human myelomas and proposed a paracrine model where the primary source for IL-6 was monocytes and myeloid cells [160]. In support of the autocrine hypothesis, Schwab and colleagues showed that the addition of neutralizing anti-IL-6 monoclonal antibody or IL-6 antisense oligonucleotides can inhibit proliferation of the human myeloma cell line, U266, and that these effects could be reversed by adding IL-6 [161]. In U266 cells, extremely low amounts of IL-6 are sufficient for allowing an autocrine growth. A possible autocrine role for IL-6 has also been reported for Burkitt lymphoma, non-Hodgkin's lymphomas, chronic lymphocytic leukemias and acute myeloid leukemias [162–165]. Kurzrock presented evidence that IL-6 levels are elevated in both relapsed and newly diagnosed Hodgkin's and non-Hodgkin's lymphoma and that these levels correlate with established prognostic features [166].

Although initially reported to induce tumor necrosis [167], TNF-α was subsequently shown to promote the proliferation and survival of some tumor cell lines [168–170]. Liu and coworkers presented evidence that activation of p38 MAP kinase is required for TNF-α-supported proliferation of leukemia and lymphoma cell lines [171]. TNF-α can also act as an autocrine factor for growth of myeloma cells by stimulating IL-6 production [172]. *In vivo* studies have also shown that TNF-α can stimulate tumor growth in experimental animal models. Kalliriowski and coworkers demonstrated that low doses of recombinant TNF-α increased tumor growth in a rat carcinosarcoma model by increasing tumor blood flow [173]. Gelin and associates obtained indirect evidence that endogenous TNF production contributed to tumor growth by showing that pretreatment of mice bearing a methylcholanthracene-induced sarcoma with anti-TNF antibody inhibited tumor growth [174]. Clinical studies suggest that TNF-α and p55 soluble receptor plasma levels predict treatment outcome in lymphoma patients [175 and 176].

Interleukin-8 is constitutively produced by a variety of human carcinoma cell lines [177, 178] and may act as an autocrine growth factor. IL-8 has been shown to induce proliferation of melanoma cells [179–181]. Melanoma cells secrete significant amounts of bioactive IL-8 protein into the culture supernatant, and this secretion is further augmented by IL-1 and PMA treatment [179]. Exposure of some human melanoma cell lines *in vitro* to antisense oligonucleotides targeted against two different sites of human IL-8 mRNA inhibited secretion of IL-8 protein into the culture medium and blocked cell proliferation and growth in soft agar in a dose-dependent manner. Two IL-8-specific monoclonal antibodies were also demonstrated to block melanoma cell proliferation. Gutman and coworkers demonstrated the significant role of the local tumor microenvironment on IL-8 production by melanoma cells [182]. IL-8 may also serve as an autocrine growth factor for human colorectal carcinoma [183 and 184], pancreatic carcinoma [185] and mesothelioma cells [186].

8 Inflammatory cytokines and cancer cachexia

About half of all cancer patients experience a wasting syndrome called cachexia, a complex syndrome characterized by anorexia, weight loss, asthenia, muscle weakness and catabolism (reviewed in [187]). Pro-inflammatory

cytokines, such as TNF-α, IL-1, and IL-6 have a prominent role in the pathogenesis of cancer cachexia. They can elicit many but not all host changes seen in cancer cachexia, including loss of appetite, loss of body weight, and the induction of acute-phase protein synthesis.

The role for TNF-α was initially suggested by studies on cachexia in rabbits with chronic parasitic (*Trypanosoma brucei*) infections [188]. These studies led to the identification of a macrophage-derived protein (cachectin) that suppressed lipoprotein lipase activity in mice and in 3T3-L1 adipocytes *in vitro*. Cachectin was purified from a murine macrophage cell line and shown to be identical to TNF-α [189]. *In vivo* studies have shown that continuous prolonged infusion of recombinant TNF-α is required for progressive weight loss and cachexia [190]. Nude mice injected intramuscularly with TNF-α-secreting Chinese hamster ovary (CHO) cells develop progressive weight loss, anorexia, lipid depletion [191], as well as selective suppression of erythropoiesis, albumin synthesis, bone resorption and hypercalcemia [192, 193]. The most convincing experimental data for the role of TNF-α in cancer cachexia has been provided by Yoneda et al. [194]. A paraneoplastic syndrome of hypercalcemia, weight loss and leucocytosis, induced by a squamous cell tumor of the maxilla in patients, was reproduced by human tumor xenografts in nude mice and reversed by the administration of anti-TNF-α antibody.

Another interesting candidate for inducing cachexia is IL-6. The effects of IL-6 *in vivo* were assessed by inoculating nude mice with CHO cells that had been transfected with murine IL-6. Only those mice inoculated with the IL-6 gene-transfected CHO cells demonstrated a number of paraneoplastic syndromes including hypercalcemia, cachexia, leukocytosis, and thrombocytosis [195]. Both the injection of IL-6 in mice and the treatment of 3T3-L1 adipocytes with IL-6 reduced heparin-releasable and tissue lipoprotein lipase activity in a dose-dependent manner [196].

Interleukin-1 has also been proposed as a mediator of cachexia [197]. IL-1, like TNF-α, can cause increased hepatic uptake and muscular release of amino acids, an acceleration of whole-body protein turnover, and an increased degradation and depletion of muscle protein [198]. Specific neutralization of individual cytokines in animal models of wasting has suggested the therapeutic validity of the anticytokine approach, while also revealing that no single cytokine is responsible for all of the abnormalities contributing to cachexia [199].

9 p38 MAP kinase expression and activation in cancer

Stimulation of p38 MAP kinase activity occurs following dual phosphorylation on Threonine-180 and Tyrosine-182 in the p38 activation loop which causes a conformational change that exposes the enzyme active site [106, 200]. The p38α gene is ubiquitously expressed [201]. Activated p38 MAP kinase appears to be very commonly expressed in a constitutive manner in a broad range of human cancers including non-small cell lung (NSCLC) [201, 202], breast [150, 203, 204], gastric [155] and colorectal [205] carcinomas and malignant melanoma [154]. Using Western blot analysis of tissue homogenates from resected NSCLC and matched non-neoplastic lung tissue, Greenberg and associates determined that activated p38 was consistently increased in tumor compared to normal tissue [202]. Contrary to their expectations, ERK and JNK, the MAPK pathways traditionally associated with cell growth and perhaps malignant transformation, were not consistently activated in the human lung tumor samples. However, p38, a MAPK usually associated with stress responses, growth arrest, and apoptosis, was activated in all of the human lung cancer samples, suggesting an additional role for this pathway in malignant cell growth or transformation.

Several investigators have looked at the expression of p38 MAP kinase in breast cancer. Xiong and colleagues noted that heregulin, a member of a family of polypeptide growth factors that bind to receptor tyrosine kinases ErbB3 and ErbB4, activated extracellular signal-regulated protein kinases, Akt kinase, and p38 MAP kinase [204]. In investigating downstream signaling pathways involved in heregulin-mediated up-regulation of VEGF, they noted that only the selective inhibitor of p38 MAP kinase (SB203580), not the extracellular signal-regulated kinase inhibitor PD98059 nor the inhibitor of phosphatidylinositol 3-kinase-Akt pathway (Wortmannin), blocked the up-regulation of VEGF by heregulin. These results show that heregulin can activate p38 MAP kinase to enhance VEGF transcription *via* an upstream heregulin response element, leading to increased VEGF secretion and angiogenic response in breast cancer cells. Salh et al. showed that aberrant mitogenic signaling in human breast cancer *in vivo* involves p21-activated kinase (Pak), p38 MAP kinase, and MAPKAPK2 [203]. They proposed that this pathway may serve as a useful targeting nexus for investigation of small molecule inhibitors in human breast cancer. Chen and associates noted that endogenous p38 MAP kinase activity is elevated in invasive breast cancer cells and that con-

stitutive p38 MAP kinase activity is important for over-production of urokinase plasminogen activator in these cells and maintaining the invasive phenotype [150, 206]. They suggested that α_v integrin was responsible for maintaining elevated p38 MAP kinase activity and urokinase plasminogen activator expression in invasive tumor cells. Integrin ligation specifically induced p38 MAPK activation and urokinase plasminogen activator up-regulation in invasive MDA-MB-231 cells but not in non-invasive MCF-7 cells. Similarly, Jung and colleagues suggested that the p38 MAP kinase pathway is involved in urokinase plasminogen activator expression and matrix invasiveness in gastric cancer cells [155].

Miki and coworkers assessed the activation of p38 MAP kinase and ERKs in human colorectal adenocarcinoma by immunoblotting with antibodies raised against each activated form [205]. They also assessed the alteration of proliferative and apoptotic states, and analyzed the association of p38 MAP kinase with these alterations. They found that p38 MAP kinase was constitutively activated and was associated with increased proliferative and apoptotic states in colorectal cancers.

10 The p38 MAP kinase connection in cancer

Cells in a multicellular organism need to communicate with each other in order to control their growth and development, and to coordinate their functions. The extracellular signals involved in these processes generate different types of responses, which depend on specific intracellular mechanisms, slow and long-lasting, or rapid and transient.

Among the signaling cascades involved in the response of cells to growth factors, cytokines, or environmental stress, are those which activate the so-called MAP (mitogen-activated protein) kinase family of kinases. The p38 signaling transduction pathway, a MAP kinase pathway, plays an essential role in regulating many cellular processes including inflammation, cell differentiation, cell growth and death [106, 200, 201]. It was originally identified as a serine/threonine kinase activated by stimulation of monocytes with bacterial lipopolysaccharide (LPS) and was later shown to regulate LPS-induced production of the proinflammatory cytokines TNF-α and IL-1β [103]. Overproduction of these cytokines has been implicated in a wide variety of diseases with an inflammatory component, such as rheumatoid arthritis, endo-

toxic shock, inflammatory bowel disease, and many others. Activation of p38 is induced by environmental stresses such as osmotic shock and UV light, and by proinflammatory stimuli such as LPS, IL-1β, and TNF-α. Activation of p38, often through extracellular stimuli such as bacterial pathogens and cytokines, mediates signal transduction into the nucleus to turn on the responsive genes.

MAPKAP kinase2 (MK2) is one of several kinases that are regulated through direct phosphorylation by p38 MAP kinase. Following phosphorylation of MK2, Ben-Levy and coworkers showed that nuclear p38 is exported to the cytoplasm in a complex with MK-2 [207]. The cytoplasm translocation of MK2 requires phosphorylation by p38 without a requirement for MK2 activity. MK2 serves both as an effector of p38 by phosphorylating substrates and as a determinant of cellular localization of p38. The authors speculated that nuclear export of p38 and MK-2 may permit them to phosphorylate substrates in the cytoplasm such as eukaryotic initiation factor (eIF)-4E and PHAS-1. By introducing a targeted mutation into the mouse MK2 gene, Kotlyarov et al. determined the physiological function of MK2 in vivo [208]. Mice that lack MK2 show increased stress resistance and survive LPS-induced endotoxin shock. This is due to a reduction of approximately 90% in the production of TNF-α and not to a change in signaling from the TNF receptor.

The significant role of the inflammatory response regulated by p38 MAP kinase in facilitating tumor growth has been described in earlier sections of this Chapter and is summarized in Table 2. TNF-α may serve as an important kingpin molecule in setting the stage to support the proinflammatory microenvironment of the tumor. Tumors produce MCP-1, which recruits macrophages to the tumor site [96]. Interaction with tumor cells serves as a stimulus to induce TNF-α production by these tumor-infiltrating macrophages [209]. TNF-α can further up-regulate production of MCP-1 [101], along with potent angiogenic factors VEGF, IL-8, and b-FGF, through activation of transcription factors [102]. The production of TNF-α, IL-1, IL-6, IL-8, MCP-1 and GM-CSF and several functional activities of these cytokines are regulated by p38 MAP kinase [106]. These proteins are central mediators of the inflammatory response. Similarly, the effects of these proinflammatory cytokines, including induction of angiogenesis, induction of proteases involved in tumor invasion, and expression of adhesion molecules in tumor cells and endothelial cells, are regulated by p38 MAP kinase. This cascade of cytokines and the resultant inflammatory microenvironment regulated by

Table 2.
Some potential mechanisms for antitumor activity of p38 MAP kinase inhibitors.

1)	Blocking tumorigenesis by inhibiting local production of endogenous promoters, such as TNF-α [59, 60]
2)	Inhibition of autocrine and paracrine cytokine growth factors (TNF-α, IL-6 and IL-8; primarily for myeloma, leukemia, and lymphoma) [157]
3)	Inhibition of local MCP-1 production in tumor microenvironment which regulates macrophage tumor content [104]
4)	Blocking stromal proliferation
5)	Inhibition of production of cascade of tumor angiogenic factors [102]
6)	Induction of apoptosis in certain tumor cell lines [211, 212]
7)	Inhibition of metastasis by preventing invasive phenotype of cells and production of urokinase-type plasminogen and MMPs [150–152]
8)	Inhibition of metastasis by preventing attachment of metastatic cells to distant vasculature [142–145]
9)	Regulation of cyclooxygenase-2 (COX-2) production in tumor cells and macrophage [213, 214]
10)	Suppression of tumor-associated cachexia by inhibiting inflammatory cytokine production [199]
11)	Potentiate antitumor activity of radiation and radiomimetic drugs by blocking G2 cell cycle delay during DNA repair [215]
12)	Modulate the transition of breast cancer growth from steroid-hormone to growth-factor dependence [216]

p38 MAP kinase has impact on various stages involved in tumor development and the metastatic process (Fig. 1). However, it must be pointed out that some antitumor activities, such as taxol-induced apoptosis of breast cancer cells, may be dependent on the p38 MAP kinase pathway [210]. Other studies suggest that either p38 MAP kinase is not involved in promoting apoptosis or is protective against this process [211, 212, 217]. The potential for inhibition of the production and signaling of multiple proinflammatory cytokines and, consequently, the downstream cascades induced by these cytokines, is a potential advantage for small molecule p38 MAP kinase inhibitors in the treatment of cancer over strategies that inhibit the production of, antagonize or neutralize individual cytokines, such as anti-TNF antibodies [218, 219]. It is interesting that the TNF-α has such diametrically opposed functions – inducing tumor necrosis on one hand and stimulating tumor development on the other. The history of TNF research in cancer demonstrates how essential it is in science to keep an open mind and to constantly adjust one's view to new findings and, if necessary, to revise old and dearly loved dogmas.

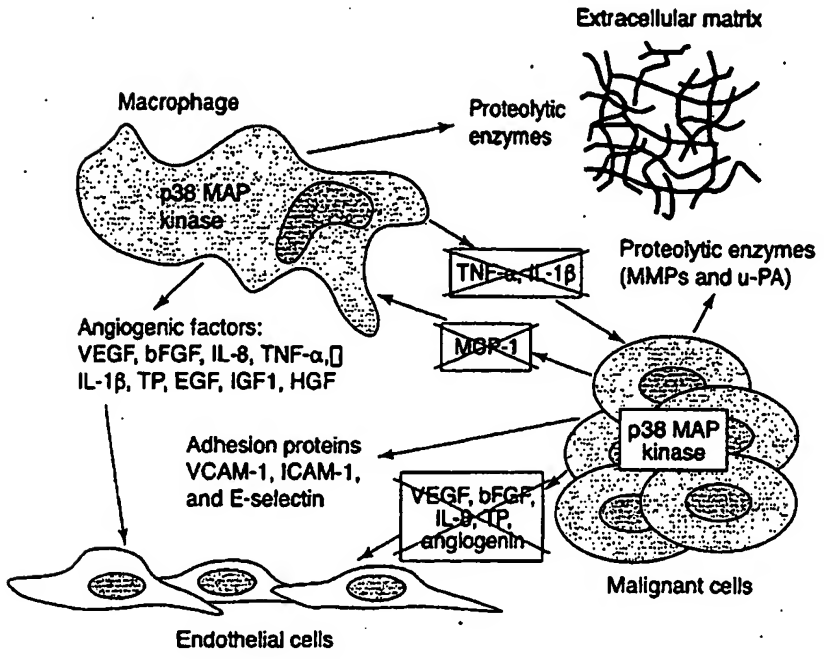

Fig. 1.
p38 MAP kinase can have multiple effects on tumor development and progression, ranging from regulation of angiogenic factors, growth factors, proteolytic enzymes for tumor invasion, and adhesion proteins for tumor metastasis.

11 Concluding remarks

Animal models demonstrate experimentally that chronic sterile inflammation predisposes to the development of various forms of cancer. For example, intraperitoneal administration of nondigestible, nongenotoxic mineral oils, such as pristane, or plastic discs in BALB/c mice promotes the formation of chronic granulomatous tissue and induces the high incidence of B lymphocytic (plasma cell) tumors [220]. In these animal models, the tumors generally arise in the inflammatory tissue, indicating that local inflammatory mediators are responsible for their development.

A good example on the involvement of chronic inflammation in human cancer is illustrated through inflammatory bowel disease and colon carcinogenesis. Patients with either chronic ulcerative colitis or Crohn's disease have a five- to seven-fold increased risk of developing colorectal carcinoma [221].

It is generally thought that the colitis must persist for at least 8 years to significantly increase the risk of cancer [222]. Cancer generally appears after a median duration of approximately 15 years. The longer the inflammation persists, the higher the risk of associated carcinogenesis [223]. Acute inflammation, such as occurs in response to a transient infection, is not regarded as a risk factor for the development of neoplasia. Chronic use of anti-inflammatory drugs decreases the incidence of colon carcinogenesis associated with inflammatory bowel diseases (reviewed in [223]).

Nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both the COX-1 and COX-2 protein, the two enzymes that convert arachidonic acids to prostaglandins. Significant antitumor effects of NSAIDs appear to derive from their inhibition of COX-2 [222, 223]. COX-2 is expressed during inflammation and is primarily expressed by inflammatory monocytes and macrophages. Selective COX-2 inhibitors, such as celecoxib (SC-58635) and NS-398, suppress azoxymethane-induced colon carcinogenesis in rats [224]. Drastic reduction in polyp number results from COX-2 gene knockout as well as from selective COX-2 inhibition in a mouse model of human familial adenomatous polyposis. COX-2 is highly inducible by proinflammatory cytokines and highly expressed in human colon carcinoma, squamous cell carcinoma of the esophagus, and skin cancer. Selective inhibitors of p38 cause a depression in COX-2 expression in LPS-treated macrophages and tumor cells [213, 214], providing an additional mechanism for antitumor activity by p38 inhibitors. Similarly, Fan and colleagues demonstrated in human gastric cancer cells that IL-1β upregulates COX-2 gene expression through the activation of p38 MAP kinase [214].

As early as 1844, French doctors applied gauze dressings soaked in gangrenous tissue to small incisions in tumors, concluding that "gangrene seems to have replaced live cautery, caustics or the scalpel" [225]. Distressed by the death of a young patient with a bone sarcoma, William B. Coley, a New York surgeon, studied departmental case records and discovered a patient who had been cured of a thrice recurrent sarcoma after two attacks of erysipelas, a subcutaneous streptococcal infection. By the end of the 19th century, Coley introduced the idea that tumor regression in human cancer patients could be accomplished by challenging the tumor with bacterial toxins [226]. Much later, Carswell and colleagues identified a protein in the serum of endotoxin-treated rabbits, which was responsible for the hemorrhagic necrosis of tumors [227]. It was then named tumor necrosis factor (TNF) and later TNF-α after

the discovery of lymphotoxin or TNF-β [228]. Although the historical background and preclinical data appeared promising, systemic therapy with recombinant-derived TNF-α in human cancer has proven highly toxic and showed a disappointing response rate against all tumor types tested to date [229, 230]. Local therapy, particularly limb perfusion, has produced encouraging response rates, although there has been no effect on survival [231].

TNF-α, on the other hand, may also contribute to tumor progression. TNF-α can be detected in malignant and/or stromal cells in human ovarian, breast, prostate, bladder, and colorectal cancer, lymphomas, and leukemias, often in association with IL-1 and IL-6 and macrophage colony-stimulating factor [1]. TNF-α appears to be a kingpin molecule for evoking the strategy of inhibiting p38 MAP kinase and effects a wide range of effects that promote tumor growth, including activating enzyme cascades that may enhance tumor spread, stimulate angiogenesis, induce proliferation in certain hematopoietic cancer, and increase adherence of tumor cells to endothelial surfaces. It may have a pivotal role in orchestrating the expression of other TNF-inducible cytokines (e.g., IL-1β, IL-6, IL-8, MCP-1 and GM-CSF) in cancer. TNF-α can also contribute to tumor progression by inducing DNA damage and inhibiting DNA repair by a nitric oxide-dependent mechanism [232]. It can also stimulate bone resorption and inhibit synthesis of proteoglycan in cartilage [233, 234]. It can contribute to the generation of tumor stroma by its procoagulant activities [235], and by enhancing fibroblast proliferation and chemotaxis [236, 237]. In ovarian cancer, TNF mRNA is found in epithelial tumor islands, where there is a positive correlation with tumor grade [238]. In ovarian tumor biopsies, TNF co-localizes with MMP-9 in tumor-associated macrophages. TNF-α has been proposed to have an autocrine or paracrine role in promoting the growth and invasion of advanced human ovarian cancer [239]. In prostate cancer, tumor cell TNF production correlates with loss of androgen responsiveness [1, 240]. In non-Hodgkin's lymphoma, myelogenous leukemia and chronic lymphocytic leukemia, high circulating levels of TNF-α and its soluble receptors are associated with poor prognosis [241].

Less than a decade ago the kinases constituting mammalian MAP kinase pathways were identified through intense efforts to understand the molecular events underlying the cellular responses to extracellular signals. P38 MAP kinase is an attractive target for drug development due to its important role in controlling production and activities of various inflammatory cytokines,

including TNF-α. In recent years, a number of small-molecule orally active p38 MAP kinase inhibitors have been described in the literature [106, 242–249]. Recent studies have determined that SB203580 and other pyridinyl-imidazole inhibitors of p38 MAP kinase function by competitive binding in the ATP pocket [250–252]. The potential role of p38 MAP kinase inhibitors in the management of cancer is just beginning to emerge.

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